AMENDMENTS TO THE SPECIFICATION

Please amend the "BRIEF DESCRIPTION OF THE DRAWINGS" as follows:

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the invention will be described in relation to the drawings in which:

Figure 1. Domain organization of Rat Nedd4.

Figure 2. Protein sequence of Clone 7.7, the homolog of human clone KIAA0313.

Figure 3A. Schematic Diagram of GRF4.

Figure 3B-F. Shows the nucleic acid molecule that is [SEQ ID NO:1] and the polypeptide that is [SEQ ID NO:2]. In a preferred embodiment, the figures shows GRF4.

Figure 4A. Protein sequence alignment of CDC25 domains from several RasGEF/GRF including GRF4. The CDC25 domain of human GRF4 (hGRF4) was aligned with those of *Drosophila* GRF4 (dGRF4 [SEQ ID NO:7], identified from genomic DNA sequence [Accession number. AC005285, nucleotide sequence 122129-174319]), human Epac (hEpac), mouse RasGRF2 (mRasGRF2) [SEQ ID NO:9], *Drosophila* SOS (dSOS) [SEQ ID NO:10] and RasGRP (hRasGRP) [SEQ ID NO:11]. The three structurally conserved regions present in CDC25 domains are lighter. Both hGRF4 and dGRF4 contain a unique insertion shown in blue. Alignments were created using the program Clustal W(1.7).

Accession numbers.

hGRF4 (AB002311), dGRF4(AC005285), hEpac(AF103905), mRasGRF2(U67326), dSOS(M83931), hRasGRP(AF106071), rLin-7-C(AF090136), hPTP-BAS-1(D21209), hDlg(U61843), hPRKAR1B(M65066), hPSD-95 (AF156495), hPKGII(CAA76073), mEAG(U04294).

~~**Figure 4B.** Comparison of CDC25 domain of GRF4 with RasGRF2 revealing the insert in GRF4.~~

Figure 5. Protein sequence of alignment of Ras GRF4-REM domain including CDC25 [SEQ ID NO:12], Sos_mouse [SEQ ID NO:13], GRF2_mouse [SEQ ID NO:14], RasGEF_aimless [SEQ ID NO:15].

Figure 6A. Overall structure comparison between GRF4 and other known mammalian GRFs/GEFs which activate Ras.

Figure 6B. An example of the most well known Ras signaling pathway.

Figure 7. Sequence alignment of GRF4-PDZ domain. The PDZ domains of hGRF4 and dGRF4 [SEQ ID NO:16] were aligned with those of rat Lin-7-C (rLin-7-C) [SEQ ID NO:19], human PTP-BAS type 1 (hPTP-BAS-1) [SEQ ID NO:17], human Dlg (hDlg) [SEQ ID NO:20] and human PSD-95 (hPSD-95) [SEQ ID NO:18]. The sequences corresponding the GLGF motif present in prototypic PDZ domains are lighter.GRF4 Alignments were created using the program Clustal W(1.7).

Figure 8. Sequence alignment of GRF4-cNMP-BD. The cNMP-BD of hGRF4 was aligned with those of dGRF4 [SEQ ID NO:21], hEpac [SEQ ID NO:22], human cAMP-dependent protein kinase regulatory subunit type 1b (hPRKAR1B) [SEQ ID NO:23], human cGMP dependent protein kinase (hPKGII) [SEQ ID NO:24], and mouse cyclic nucleotide gated potassium channel (mEAG) [SEQ ID NO:25]. The conserved motifs RAA present in hPRKAR1B and hEpac that confers cAMP binding specificity are shaded in blue. The conserved motifs RTA present in hPKGII and mEAG that confers cGMP binding specificity are lighter. Alignments were created using the program Clustal W(1.7).

Figure 9. Protein sequence alignment of GRF4-RA domain including dgk-1a_ce [SEQ ID NO:26] and RalGDS_h [SEQ ID NO:27].

Figure 10. Tissue Distribution of GRF4.

Figure 11. Co-precipitation of endogenous Nedd4 in Hek 293T cells by a GST-fusion protein of the C-terminal last 150 aa of GRF4 which contains the two PY motifs .

Figure 12. Co-immunoprecipitation of GRF4 with endogenous Nedd4 in Hek 293T cells transiently transfected with Flag-tagged GRF4.

Figure 13. Method used for the *in vitro* GEF assay.

Figure 14. *In vitro* GEF assay using immunoprecipitated full-length GRF4 demonstrating activation of Ras by GRF4 (additional data in Fig. 23(e)).

Figure 15. GRF4 forms stable complex with GST-Ras in vitro.

Figure 16. GRF4 induces foci formation in Rat2 fibroblasts.

Figure 17. GST-fusion protein of GRF4-PDZ domain binds full-length GRF4 expressed in Hek 293T cells.

Figure 18. Biotinylated peptide of the last 15 amino acid sequence of GRF4 containing a PDZ-binding motif (SAV*) binds full-length GRF4.

Figure 19 (a), (b) and (c) shows the ~~Nucleic~~ nucleic acid molecule sequence of [SEQ ID NO:1] and Figure 19(a) shows the amino acid sequence of [SEQ ID NO:2]. The

Figures 19 (d) and (e) ~~The figure~~ shows the nucleic acid molecule sequence that is [SEQ ID NO:3] and amino acid sequences [SEQ ID NOS:4,5,6]. In a preferred embodiment, [SEQ ID NO:3] is the Clone 7.7 DNA nucleic acid molecule sequence

Figure 20. Plasma membrane localization of GRF4.

Figure 21. GRF4 domain organization and expression. (a) GRF4, depicting its cNMP (cAMP/cGMP) binding domain (cNMP-BD), a Ras Exchange Motif (REM), a PDZ domain, a Ras Association (RA) domain, a CDC25 domain which contains an insert region (white box) and a C terminus which includes 2 PY motifs (PPxY) that bind Nedd4 WW domain(s). The COOH terminus ends with the sequence SAV, conforming to a PDZ binding motif. Sequence alignment of the CDC25, cNMP-BD and PDZ domains is provided in the Supplementary material.

(b) Northern blot analysis of GRF4 mRNA in multiple regions of human brain, probed with the radiolabelled cDNA corresponding to the 3' region of human GRF4 (nucleotides 4286-4620 of KIAA0313), and depicting expression of ~7.5 and ~8.5 kb size transcripts. (blot purchased from Clontech). A multiple rat tissue Northern blot (from Clontech) probed with GRF4 cDNA revealed strong expression primarily in the brain (not shown).

(c) Western blots depicting characterization of anti GRF4 antibodies and expression of the GRF4 protein in synaptosomes. Polyclonal anti GRF4 antibodies were raised against a GST-fusion protein encompassing the C terminus (amino acids 1350-

1499) of GRF4, and recognize the ~180 kDa GRF4 protein either heterologously expressed in HEK-293T cells (epitope-tagged with HA, Flag (Fl) or myc tags) (left panel), or endogenously expressed in synaptosomes from adult (Ad) or embryonic (Emb) rat brain (right panel). No protein was detected with the pre-immune (pre-imm) serum. tfxn, transfection; IP, immunoprecipitation; αGRF4, anti GRF4 antibodies.

Figure 22. Binding of cAMP to the cNMP- binding domain (cNMP-BD) of GRF4.

(a) *In vitro* binding of GST-GRF4-cNMP-BD, but not GST alone, to immobilized cAMP. cAMP-agarose beads were incubated with soluble GST-GRF4-cNMP-BD or GST alone, washed, proteins separated on 10% SDS-PAGE and immunoblotted with anti GST antibodies (upper panel). Total amount of proteins incubated with the cAMP beads is shown in the lower panel (coomassie).

(b) Precipitation of transfected GRF4, but not mutant GRF4 lacking its cNMP-BD (ΔcNMP-BD), with cAMP agarose beads. cAMP agarose beads were incubated with cell lysates from HEK-293T cells expressing either GRF4 or ΔcNMP-BD, followed by washing of beads, SDS-PAGE, and immunoblotting with anti GRF4 antibodies (upper panels). Expression of full length and mutant GRF4 was verified by immunoblotting aliquots of the respective cell lysates with the same antibodies (bottom panels). Right and left panels in (b) represent two separate experiments.

Figure 23 cAMP/cGMP-mediated activation of Ras, but not Rap1, by GRF4 in living cells. (a) cAMP-dependent and PKA-independent activation of ras by GRF4.

HEK-293T cells were transfected (or not) with Flag-tagged GRF4, serum-staved overnight, pre-treated (or not) with the PKA inhibitors H-89 (10 mM) or Rp-cAMPS (50 mM) for 30 min., and then treated (or not) with the cAMP analogue 8-Br-cAMP (500 _ M) for 15 min. Cells were then lysed and lysate incubated with immobilized Ras binding domain (RBD) of Raf1 (GST-Raf1-RBD), which binds activated (GTP-bound) Ras. Co-precipitated activated ras was then detected with anti Ras antibodies (Quality Biotech) (upper panel). Lower 2 panels depict the amounts of total endogenous Ras and of the transfected GRF4 (detected with anti Ras and anti Flag antibodies, respectively).

(b) cGMP-dependent and PKG-independent activation of ras by GRF4.

Cells were transfected (or not) with Flag-GRF4 and serum-starved overnight as in (a) above, pre-treated (or not) with the PKG inhibitors H-8 (5 mM) or Rp-cGMPS (25 μM) and then treated (or not) with the cGMP analogue 8-Br-cGMP (500 mM), as in (a) above. Activated Ras was then precipitated with GST-Raf1-RBD (upper panel), as in (a). Lower panels show total endogenous Ras and GRF4 expressed in the cells.

(c) Activation of Ras via GRF4 following elevation of intracellular levels of cAMP or cGMP.

Cells were transfected as in (a), and were then treated (for 15 min) with either Forskolin (50 mM) plus the cAMP phosphodiesterase inhibitor IBMX (100 mM), to elevate intracellular cAMP, or with YC-1 (100 mM) plus the cGMP phosphodiesterase inhibitor DiPy (10 mM), to elevate intracellular cGMP. Parallel treatments with 8-Br-cAMP or 8-Br-cGMP were used as positive controls. Lysates of treated cells were then incubated with GST-Raf1-RBD to

Please replace the paragraph on page 9, lines 8-16 with:

--GRF4 was isolated as a PY (xPPxY) motif-containing polypeptide. A 450 nucleotide murine fragment encoding two PY motifs was initially isolated. At the amino acid level this fragment was 75% identical (95% similar) to the hypothetical gene product of the human Genbank entry KIAA0313. We characterized the human polypeptide, which we named GRF4 (also known as Ras GRF4 or CNRas GEF), because it is a fourth class of Ras guanine nucleotide exchange factor (GEF). GRF4 polypeptides were unknown prior to this invention. The hypothetical polypeptide based on KIAA0313 DNA sequence information cannot predict if a polypeptide is translated, its sequence, activity or the extent of post-translational modifications.--

Please replace the last paragraph on page 10 with:

--GRF4 activity and effects on Ras and Rap1 GRF4 is activated by distinct signaling pathways that involve a G-coupled receptor signaling pathway (Fig. 19 A-E). GRF4 can be activated by a G-protein coupled receptor via an association of GRF4-PDZ domain and its binding motif present in many such receptor. This activation process depends on the activation state of the receptor. Binding of GRF4 to such a receptor leads

to activation of GRF4 as a result of conformational changes or membrane recruitment of GRF4 (or [BOTH).] In one of the aspects of the inventions, activation of a [G-COUPLED] receptor leads to elevation of cAMP which modulates GRF4 activity by directly binding to GRF4-cAMP-BD. The SAV* motif of GRF4 can be involved in an intramolecular interaction with GRF4-PDZ domain and this interaction may have regulatory roles in GRF4 activity. Likewise, this motif can bind to other PDZ-containing proteins associating with the plasma membrane. GRF4 binds preferentially to nucleotide-free and GTP-bound Ras. The RA domain of GRF4 mediates GRF4 binding to Ras-GTP. In so doing, GRF4 functions as a downstream Ras effector. The ubiquitin protein ligase Nedd4 interacts with GRF4 through WW domain-PY motif interaction and ubiquitinates GRF4 and targets it for degradation.—

On page 10, please replace the paragraph between lines 11-21 with:

--CDC25 Domains GRF4 harbours a central catalytic region called CDC25 domain, named for the prototypic Ras activator in Saccharomyces cerevisiae (21), from which the function of GRF4 was deduced.- CDC25 domains catalyze guanine-nucleotide exchange/release activity on Ras family GTPases. The CDC25 of GRF4 is 48-52% similar to those of yeast CDC25, SOS and RasGRF/RasGRF2. Fig. 4 shows the alignment of CDC25 domains from various proteins including GRF4. From the mutagenesis studies of yeast CDC25, several conserved arginine residues were proposed to be critical for its activity (22). These conserved arginine residues are also conserved in GRF4. Similar to CDC25, SDC25, RasGRF1/2 and SOS, GRF4 contains blocks of highly conserved sequences (Fig. 4A) which were recently demonstrated, based on the tertiary structure of SOS bound to Ras, to play a critical role in the activity of the CDC25 domain towards Ras (23). However, unique to GRF4, the GRF4-CDC25 domain also contains an insert (about 40 amino acids) not found in SOS, RasGRF2 or other RasGRF3 (Fig. 3B-F).--

On page 20, please replace the paragraph between lines 2-17 with

--The invention also includes oligonucleotide probes made from the [CLONED] GRF4 nucleic acid molecules described in this application or other nucleic acid

molecules of the invention, such as Clone 7.7 (SEQ ID NO: 28) (see [MATERIALS] and methods section). The probes may be 15 to 30 nucleotides in length and are preferably at least 30 or more nucleotides. A preferred probe is at least 15 nucleotides of GRF4 in [SEQ ID NO: 1] or the Clone 7.7 sequence (SEQ ID NO: 28). The invention also includes at least 30 consecutive nucleotides of [SEQ [ID NO:] 1] or the Clone 7.7 sequence. The probes are useful to identify nucleic acids encoding GRF4 peptides, polypeptides and polypeptides other than those described in the application, as well as peptides, polypeptides and polypeptides functionally equivalent to GRF4. The oligonucleotide probes are capable of hybridizing to the sequence shown in [SEQ ID [NO:] [1]] under stringent hybridization conditions. A nucleic acid molecule encoding a polypeptide of the invention may be isolated from other organisms by screening a library under moderate to high stringency hybridisation conditions with a labeled probe. The activity of the polypeptide encoded by the nucleic acid molecule is assessed by cloning and expression of the DNA. After the expression product is isolated the polypeptide is assayed for GRF4 activity as described in this application.

On page 42, please replace the paragraph between lines 3-7 with

--Among the positive clones isolated was Clone 7.7 (SEQ ID NO: 28). Clone 7.7 (SEQ ID NO: 28) is a novel protein, the partial amino sequence of which exhibits 75% identity and 95% similarity of that of the novel human brain cDNA called KIAA0313. (amino acid nos. 1348-1499 of SEQ ID NO: 2) Because of this remarkable high sequence similarity between them, Clone 7.7 (SEQ ID NO: 28) is the mouse homologue of KIAA0313 (amino acid nos. 1348-1499 of SEQ ID NO: 2) and obtained the full-length cDNA of KIAA0313.---